UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005853

APR 24 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

RANDUM

ECT: Ronilan Fungicide - EPA Registration No. 7969-53

Tox Chem. No. 323C

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ested Action:

Review an additional mutagenicity study for Ronilan clozolin) and assign Accession and MRID Numbers.

mmendation:

The CHO/HGPRT assay is acceptable and provides evidence vinclozolin is not mutagenic. However, several deviations accepted procedures for this assay were noted, i.e., no ent controls and no assessment for cytotoxicity at the time utant selection, therefore, it is recommended that these

1, 19

deficiencies be corrected in the future to avoid compromising the assay results. Additionally, a QA/GLP statement of compliance is required and should be submitted to the Agency.

Conclusions:

The Chinese Hamster Ovary (CHO)/HGPRT Forward Gene Mutation Assay is acceptable.

- Ronilan ranging from 316 to 10,000 ug/mL in the presence and absence of S9 activation did not induce a mutagenic effect in CHO cells. The assay is considered only marginally acceptable because of several deviations from accepted CHO/HGPRT assay procedures. These are: No solvent control and no cytotoxicity determination at the time of mutant selection. However, the lack of any appreciable increase in mutant colonies at any test dose with or without S9 activation in conjunction with cytotoxicity without S9 activation does suggest that the above deficiencies did not alter the outcome of the study.

  A QA/GLP statement of compliance is required. Although the assay, itself, is acceptable, a QA/GLP statement should be submitted to the Agency prior to its final acceptence.

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EPA: 68-02-4225
DYNAMAC No. 251-A
April 13, 1987

DATA EVALUATION RECORD

VINCLOZOLIN

Mutagenicity--Chinese Hamster Ovary (CHO)/HGPRT Forward Gene Mutation Assay

STUDY IDENTIFICATION: Gelbke, H.-P. and Jäckh, R. Report on a point mutation test carried out on CHO cells (HGPRT locus) with the test substance vinclozolin. (Unpublished study No. 85/352 prepared and submitted by BASF Toxikologie, Ludwigshafen, FRG; dated October 1985.) Accession No. 261082.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
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Signature: Irvin Felkner

Date: 4-13-87

3

1. CHEMICAL: Vinclozolin; 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione.

2. TEST MATERIAL: Vinclozolin from batch No. 283230 was described as a 99.5% pure white solid.

3. STUDY/ACTION TYPE: Mutagenicity--Chinese hamster ovary (CHO/HGPRT) forward gene mutation assay.

4. STUDY IDENTIFICATION: Gelbke, H.-P. and Jäckh, R. Report on a point mutation test carried out on CHO cells (HGPRT locus) with the test substance vinclozolin. (Unpublished study No. 85/352 prepared and submitted by BASF Toxikologie, Ludwigshafen, FRG; dated October 1985.) Accession No. 261082.

5. REVIEWED BY:

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7. CONCLUSIONS:

A. Under the conditions of the Chinese hamster ovary (CHO)/HGPRT forward mutation assay, four nonactivated and S9-activated doses of vinclozolin ranging from 316 to 10,000 µg/mL did not induce a mutagenic response. The highest dose was clearly cytotoxic without S9 activation and slightly cytotoxic with S9 activation. Although several deviations from accepted procedures for this assay were noted, i.e., no solvent controls and no assessment for cytotoxicity at the time of mutant selection, it was concluded that these deficiencies probably did not alter the outcome of the study.

B. The study is acceptable.

8. RECOMMENDATIONS:

In future studies, it is recommended that the above-mentioned deficiencies be corrected to avoid compromising the assay results. Additionally, a QA/GLP statement of compliance is required.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Vinclozolin from batch No. 283230 was described as a >99.5% pure white solid. The test material was stored under refrigeration and dissolved in dimethylsulfoxide (DMSO).

2. Cell Line: Chinese hamster ovary (CHO-K1) cells were obtained from Flow Laboratories, FRG. Cells were maintained as monolayers in Hams' F-12 medium supplemented with 10% fetal calf serum (FCS), 200 mM glutamine, and antibiotics. Prior to use, cultures were cleansed of 6-thioguanine (TG^r)-spontaneous mutants by growing the cells for 1 week (two subcultures) in F-12 medium containing 5×10^{-6} moles thymidine, 1X 10^{-5} moles hypoxanthine, and 9.2×10^{-6} moles aminopterine.

3. Metabolic Activation: The S9 microsomal fractions used in this assay were prepared from the livers of male Sprague-Dawley rats induced with Aroclor 1254. The S9 mix, containing 30% S9, was prepared on the day of use.

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[1]Only items appropriate to this DER have been included.

5

4. Preliminary Cytotoxicity Assay: The details of the preliminary cytotoxic assay were not reported; however, the authors stated that a dose range of 0.01 to 10,000 µg/mL was assayed with and without S9 activation.

5. Forward Mutation Assay: Duplicate cultures of precleansed cells, seeded at a density of 10^5 cells/flask, were grown for 24 hours and refed medium without FCS. Prepared cells were exposed to four doses of the test material (0.3 to 10 mg/mL), the negative control (media only), or the positive controls (ethylmethanesulfonate, EMS, at 300 µg/mL/-S9; 3-methylcholanthrene, 3-MC, at 10 µg/mL/+S9) in the absence and presence of S9 activation. The solvent (DMSO) for the test material and positive controls was not assayed.

Four hours after treatment, monolayers were rinsed, refed fresh medium, and incubated for a 12-hour recovery period; recovered cells from each dose level were pooled. The cytotoxicity assay was performed by plating duplicate aliquots of 200 cells/treatment group in nonselective medium; cells were incubated for 9 days and the cloning efficiency (CE) was determined.

The remaining pooled cells were reseeded in duplicate at 10^5 cells/flask and allowed a 9-day expression period. During the expression period, cells were periodically subcultured. For mutant selection, the cells were plated at a density of $3x10^5$ cells/plate in selective medium containing 6-TG; four replicates/dose were prepared. CE following expression was not determined. At the conclusion of a 7-day incubation period, clones were fixed, stained, and counted, and mutation frequencies (MFs) were calculated.

6. Evaluation Criteria

a. Assay Validity: The assay was considered valid if 1) the CE of the negative control was 70 to 115%; 2) CE of dose group was >10%; 3) the MF of the negative control was $>15x10^{-6}$; 4) MFs for positive controls were clearly elevated; and 5) at least four test doses ranging from noncytotoxic to cytotoxic were assayed.

b. Positive Response: The assay was considered positive if the MF of the test material exceeded the MF of the negative control by a factor of two and was accompanied by a dose-related response to increasing concentrations of the test material.

B. Protocol: A protcol was not submitted.

12. REPORTED RESULTS:

Forward Mutation Assay: Four doses of the test material (316, 1000, 3160, and 10,000 µg/mL) were evaluated in the CHO/HGPRT forward mutation assay both in the presence and absence of S9 activation. Cytotoxicity was determined following exposure, but not at mutant selection. At the highest nonactivated dose (10,000 µg/mL), 17.25% of the cells survived the 4-hour treatment. For the remaining non-activated doses, survival ranged from 38.25% at 3160 µg/mL to 74.0% at 1000 µg/mL. Under S9-activated conditions, survival at the high dose was 47.25%, or 75.3% of the media control values (survival for the media control was 62.75%). The remaining S9-activated doses were not cytotoxic when compared to the negative control.

In the mutation assay, no mutant clones were recovered for the negative controls (+/-S9), the two highest S9-activated doses (3160 and 10,000 µg/mL), and all nonactivated doses, except 3160 µg/mL. For those doses where mutant clones were recovered (316 and 1000 µg/mL/+S9 and 3160 µg/mL/-S9), average mutant yields were low (≤1.5).

Representative results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "Vinclozolin is evaluated as nonmutagenic under the test conditions applied."

B. A quality assurance statement was not provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Under the conditions of this assay, vinclozolin did not induce a mutagenic effect in CHO cells. The assay is considered only marginally acceptable because of several deviations from accepted CHO/HGPRT assay procedures.[2] These included no solvent controls and no cytotoxicity determination at the time of mutant selection. However, the lack of any appreciable increase in mutant colonies at any test dose with or without S9 activation in conjunction with cytotoxicity without S9 activation and slight cytotoxicity at an acceptable high S9-activated dose suggests that the procedural deficiencies did not alter the outcome of the study.

The ability of the test system to detect a mutagenic response was adequately demonstrated by the increased MFs calculated for the positive control groups (EMS at 300 µg/mL/-S9 and 3-MC at 10 µg/mL/+S9).

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[2] Hsie, A. W., Casciano, D. A., Couch, D. B., Karhn, D. F., O'Neill, J. P., and Whitefield, B. L. The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals. A report of the Gene-Tox program. Mutat. Res. 86 (1981): 193-214.

TABLE 1. Representative Results of the CHO/HGPRT Forward Mutation Assay with Vinclozolin

Substance	Dose (µg/mL)	S9 Activation	% Cloning Efficiency Following Exposure[a]	Average Mutant Clones[b]	Mean Mutation Frequency[c] x10^{-6}
Negative Control Medium	-	-	95.0	0	0
		+	62.75	0	0
Positive Control Ethylmethane-sulfonate	300	-	49.5	81.5	271.6[d]
3-Methylcholanthrene	10	+	63.0	4.75	15.8[d]
Test Material Vinclozolin	316[e]	-	66.25	0	0
		+	67.5	1.5	5.0
	10,000[f]	-	17.25	0	0
		+	47.25	0	0

[a] % Cloning Efficiency Following Exposure = $\frac{\text{Average number of cells recovered}}{\text{Number of cells plated (200)}} \times 100.$

[b] Average of four replicate plates.

[c] Mean Mutation Frequency = $\frac{\text{Average number of mutant clones}}{\text{Number of cells plated } (3\times10^5)}$

NOTE: Mutation Frequencies were not corrected for cytotoxicity.

[d] Positive by study authors criterion (clearly elevated mutation rates).

[e] Lowest assayed dose.

[f] Highest assayed dose; intermediate doses (3160 and 1000 µg/mL) had either no mutants or low numbers of mutants (≤ 1.25).

We conclude that the results provide sufficient evidence that vinclozolin is not mutagenic in the CHO/HGPRT assay.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-12.

APPENDIX A

Materials and Methods

Tox Rev. # 005853

Page_____ is not included in this copy.

Pages 11 through 19 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
